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(54) Title: COMPOSITIONS COMPRISING EDIBLE OILS OR FATS AND PHYTOSTEROLS AND/OR PHYTOSTANOLS SUBSTANTIALLY DISSOLVED THEREIN, METHOD OF MAKING THE SAME, AND USE THEREOF IN TREATING OR PREVENTING CARDIOVASCULAR DISEASE AND ITS UNDERLYING CONDITIONS

(57) Abstract: A composition comprises an edible oil or fat and one or more phytosterols and/or phytostanols, wherein the phytosterols and/or phytostanols are substantially completely dissolved therein by a method in which the phytosterols and/or phytostanols are heated to form a molten material which is then added to a heated oil or fat and the composition so formed is cooled to room temperature.

TITLE: COMPOSITIONS COMPRISING EDIBLE OILS OR FATS AND PHYTOSTEROLS AND/OR PHYTOSTANOLS SUBSTANTIALLY DISSOLVED THEREIN, METHOD OF MAKING THE SAME, AND USE THEREOF IN TREATING OR PREVENTING CARDIOVASCULAR DISEASE AND ITS UNDERLYING CONDITIONS

FIELD OF THE INVENTION

This present invention relates to the field of phytosterols and phytostanols and their incorporation into oils and fats.

BACKGROUND OF THE INVENTION

While recent advances in science and technology are helping to improve quality and add years to human life, the prevention of atherosclerosis, the underlying cause of cardiovascular disease ("CVD") has not been sufficiently addressed. Atherosclerosis is a degenerative process resulting from an interplay of inherited (genetic) factors and environmental factors such as diet and lifestyle. Research to date suggest that cholesterol may play a role in atherosclerosis by forming atherosclerotic plaques in blood vessels, ultimately cutting off blood supply to the heart muscle or alternatively to the brain or limbs, depending on the location of the plaque in the arterial tree (1,2). Overviews have indicated that a 1% reduction in a person's total serum cholesterol yields a 2% reduction in risk of a coronary artery event (3). Statistically, a 10% decrease in average serum cholesterol (e.g. from 6.0 mmol/L to 5.3 mmol/L) may result in the prevention of 100,000 deaths in the United States annually (4).

Sterols are naturally occurring compounds that perform many critical cellular functions. Phytosterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. The dietary source of phytosterols in humans comes from plant materials i.e. vegetables and plant oils. The estimated daily phytosterol content in the conventional western-type diet is approximately 60-80 milligrams in contrast to a vegetarian diet which would provide about 500 milligrams per day.

Phytosterols have received a great deal of attention due to their ability to decrease serum cholesterol levels when fed to a number of mammalian species, including humans. While the precise mechanism of action remains largely unknown, the relationship between cholesterol and phytosterols is apparently due in part to the similarities between the respective chemical

structures (the differences occurring in the side chains of the molecules). It is assumed that phytosterols displace cholesterol from the micellar phase and thereby reduce its absorption or possibly compete with receptor and/or carrier sites in the cholesterol absorption process.

Over forty years ago, Eli Lilly marketed a sterol preparation from tall oil and later from soybean oil called Cytellin™ which was found to lower serum cholesterol by about 9% according to one report (5). Various subsequent researchers have explored the effects of sitosterol preparations on plasma lipid and lipoprotein concentrations (6) and the effects of sitosterol and campesterol from soybean and tall oil sources on serum cholesterols (7). A composition of phytosterols which has been found to be highly effective in lowering serum cholesterol is disclosed in US Patent Serial No. 5,770,749 to Kutney et al. and comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol (beta-sitostanol). It is noted in this patent that there is some form of synergy between the constituent phytosterols, affording even better cholesterol-lowering results than had been previously achieved.

Despite the obvious and now well recorded advantages of phytosterols, not only in the treatment of CVD and its underlying conditions such as hypercholesterolemia, hyperlipidemia, atherosclerosis, hypertension, thrombosis but in the treatment of other diseases such as Type II diabetes, dementia cancer and aging , the administration of phytosterols and the incorporation thereof into foods, beverages pharmaceuticals and other delivery vehicles has been complicated by the fact that they are highly hydrophobic, water insoluble and they are also very difficult to dissolve homogenously in oils and fats. Studies have investigated how the form (for example crystalline, suspension, granular) in which the phytosterols are dosed impacts on their ability to lower serum cholesterol levels. As they are highly hydrophobic, phytosterol crystals exhibit poor solubility in the micellar phase in the digestive tract and therefore are not capable of efficiently blocking cholesterol absorption. Since solubilization of phytosterols may significantly improve the inhibition of cholesterol absorption, adaptations must be made in this respect.

Early research focused on grinding or milling the phytosterols in order to enhance their solubility (US Patent Serial Nos: 3,881,005 and 4,195,084 both to Eli Lilly). In addition, researchers have looked to the esterification of phytosterols in order to enhance their solubility. German Patent 2035069/January 28, 1971 (analogous to US Patent No. 3,751,569) describes the addition of phytosterol fatty acid esters to cooking oil. The esterification is carried out between a free sterol and a fatty acid anhydride, with perchloric acid as the catalyst. The significant drawback to this process, along with others, is the use of non-food grade catalysts and reagents.

US Patent Serial No. 4,588,717 to the David E. Mitchell Medical Research Institute describes a vitamin supplement which comprises a fatty acid ester of a phytosterol, wherein the fatty acid ester has from about 18 to 20 carbon atoms in the main carbon chain.

US Patent Serial No. 5,502,045 to Raision Tehtaat Oy AB describes the preparation of a beta-sitostanol fatty acid ester mixture. Although the attempt of this patent is to produce a soluble and stable phytostanol delivery system, there are some problems with the long term stability of these "fatty acid" esterified products due to the ultimate oxidation of the unsaturated fatty acid moiety.

US Patent Serial No. 3,865,939 to The Proctor & Gamble Company teaches edible oil compositions comprising plant sterols, in which the sterols are dissolved by way of fatty acids having length in the range of ~~from~~ six to 18 carbon atoms. This patent focuses on plant sterols as opposed to stanols the latter of which, as noted above, are inherently more difficult to solubilize in oils.

Accordingly, the provision of a method to dissolve phytosterols/phytostanols in oils and fats resulting in a product which could be used per se or incorporated without further modification into delivery vehicles would be highly desirable and has not heretofore been satisfactorily achieved.

It is an object of the present invention to obviate or mitigate the above disadvantages.

SUMMARY OF THE INVENTION

The present invention provides a method of producing an edible oil or fat composition comprising one or more phytosterols and/or phytostanols, and in which these phytosterols or phytostanols are substantially completely dissolved, which comprises:

a) heating the phytosterols and/or phytostanols to form a molten material;
b) heating the edible oil or fat;
c) mixing the molten material with the heated edible oil or fat; and
d) cooling the composition so formed.

The present invention also comprises a method of dissolving phytosterols and/or phytostanols in edible oils or fats which comprises following steps a) to d) as set out above.

The present invention further comprises an edible oil or fat composition comprising substantially completely dissolved phytosterols and/or phytostanols prepared by:

a) heating the phytosterols and/or phytostanols to form a molten material;
b) heating the edible oil or fat;
c) mixing the molten material with the heated edible oil or fat; and
d) cooling the composition so formed.

The present invention further provides foods, beverages, dietary supplements and nutraceuticals comprising an edible oil or fat composition having phytosterols and/or phytostanols substantially completely dissolved therein.

The present invention further provides a method for treating or preventing CVD and its underlying conditions including atherosclerosis, hypercholesterolemia, hyperlipidemia, hypertension, thrombosis, and related diseases such as Type II diabetes, as well as other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer by administering to an animal, such as a human, an oil or fat composition having phytosterols and/or phytostanols substantially completely dissolved therein or by administering to the animal a derivative product such as a food, beverage or nutraceutical comprising an oil or fat composition having phytosterols and/or phytostanols substantially completely dissolved therein.

What is achieved within the scope of the present invention is greatly enhanced solubility of phytosterols and/or stanols in edible oils and fats by a method which is simple, economical and which does not require extensive prior modifications to the sterols or stanols, such as by esterification. Naturally occurring and isolated phytosterols and stanols are in coarse, powder, crystalline form which is not amenable to the formation of a homogeneous mixture in water, oils or fats, without some type of modification. Although esterification of phytosterols and stanols does make them considerably more soluble in fats and oils and is a widely used technique for practical reasons, these esterified derivatives do not inhibit the absorption of cholesterol as effectively as free sterols (7b). In a number of prior patents, stanols, in particular, are esterified in order to enhance their poor solubility (see US Patent Serial No. 5,502,045 to Raision Tehtaat Oy AB). Heating beta-sitosterol in oil is described in PCT/FI99/00121; however, the resultant product is one in which the sterols are only partially dissolved.

Furthermore, within the scope of the present invention, no solvents, some of which have questionable human safety, are required to enhance dissolution of the phytosterols/stanols. The key to the success of the homogeneous dissolution, as achieved herein, is the preparation of molten or melted phytosterols that are mixed with oils or fats under appropriate conditions. This enhanced solubility in oils and fats allows the use of these media per se without any further

enhancements or modifications. Accordingly, the oil and fat compositions of the present invention can be prepared and used as such or they can be easily incorporated into foods, beverages, dietary supplements and nutraceuticals. This enhanced solubility generally translates into lower concentrations of phytosterols and/or phytostanols that need be provided in the oil or fat in order to achieve the desired therapeutic or dietary effect.

PREFERRED EMBODIMENTS OF THE INVENTION

Although the dietary and therapeutic benefits of phytosterols and phytostanols are widely recognised, a problem which has continually beset the art is the inherently non-absorbable nature of plant steroids in edible oils and fats. According to one aspect of the present invention, there is provided a method of producing a composition comprising an edible oil or a fat and one or more phytosterols and/or phytostanols, and in which these phytosterols or phytostanols are substantially completely dissolved, which comprises:

a) heating the phytosterols and/or phytostanols to form a molten material;
b) heating the edible oil or fat;
c) mixing the molten material with the heated edible oil or fat; and
d) cooling the composition so formed.

Each of the components of the composition, namely: the phytosterol or stanol and the edible fat or oil is described in more detail below. Also described are preferred or recommended procedures for achieving the desired level of steroid solubility and preferred or recommended procedures for incorporating the edible oil/fat compositions into food, pharmaceutical or nutraceutical "delivery" vehicles. Lastly, there is presented a series of non-limiting examples featuring the preparation of some compositions of the present invention.

What is achieved within the scope of the present invention, in one aspect, is the solubilization of phytosterols and more importantly phytostanols, in oils without compromising the clarity of the oils i.e. the oils so formed are clear at room temperature. In another aspect of the present invention, phytosterols and phytostanols are solubilized in animal fats that become solid at room temperature i.e. when cooled. This method allows uniform distribution of the phytosterols/stanols in the solid fat, which has not heretofore been adequately achieved, and concomitantly enhances the bioavailablity of the phytosterols/stanols.

Phytosterols/Phytostanols

As used herein, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include modified side chains also falls within the purview of this invention. It is also to be understood that, when in doubt throughout the specification, the term "phytosterol" encompasses both phytosterol and phytostanol i.e. the terms may be used interchangeably unless otherwise specified.

The phytosterols and phytostanols for use in forming derivatives in accordance with this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sunflower oil, sesame oil and fish (and other marine-source) oils. The present invention is not to be limited to any one source of phytosterols or phytostanols. US Patent Serial No. 4,420,427 teaches the preparation of sterols from vegetable oil sludge using solvents such as methanol. Alternatively, phytosterols and phytostanols may be obtained from tall oil pitch or soap, by-products of forestry practises as described in US Patent Serial No.5,770,749, incorporated herein by reference.

In one preferred form, the phytosterols/stanols which are dissolved into the oil or fat are naturally-derived or synthesized beta-sitosterol, campestanol, sitostanol and campesterol. In another preferred form, the phytosterols/stanols which are dissolved into the oil or fat are naturally-derived or synthesized sitostanol or naturally derived or synthesized campestanol or mixtures thereof.

Oils/Fats

A wide variety of edible oils and fats can be used in dissolving the phytosterols in accordance with the present invention. This includes any food-grade or nutraceutical-grade oily or fatty substance, of plant or animal or marine origin, or mixture thereof. Without limiting the generality of the foregoing, all salad and cooking oils, including sunflower oil, rapeseed oil, soybean oil, olive oil, corn oil, safflower oil, sesame seed oil may be used. Oils obtained by directed low temperature interesterification or rearrangement of animal or vegetable fatty materials, followed by removal of higher melting solids may also be used. The fats include all animal fats.

Methods of Preparation

The key feature of the method-invention as described herein is the heating of the phytosterols to form a molten material prior to mixing with the heated oil or fat. Generally, phytosterols/stanols may be heated to this molten condition at a temperature of from about 120° to 160° C, most preferably from about 135° to 145° C. Although the present invention is not so limited, the melting point of most phytosterols is about 138-140° C. In one embodiment, no additional material need be added to the molten phytosterol material. This molten material so formed is then added to oil or fat which has been previously heated to a temperature of from about 90° to 150° C, more preferably from about 100° to 120° C. The oil/fat "composition" comprising the molten phytosterols is then cooled to room temperature. The resultant product is an oil or fat in which the phytosterols are and remain substantially completely dissolved at room temperature. The colouring of the "oil" product, which is liquid, is yellow (pale or light) and transparent with no visually discernible crystal precipitate. The "fat" product is solid at room temperature.

In a preferred form, the composition of the present invention comprises from 1% to 30% phytosterol and from 99% to 70% oil or fat (hereinafter, the term % or percentage will refer to % or percentage by weight unless otherwise specified). More preferably, the composition comprises from 2% to 10% phytosterol and from 98% to 90% oil or fat. Generally, it is expected with respect to edible cooking oils, adapted as described herein in order to comprise phytosterols, that the amount of phytosterols will be 5% or less and the amount of oil 95% or greater. Alternatively, in using oils or fats to prepare delivery vehicles (foods, beverages, pharmaceutical and the like as described further below), it may be desirable to have a higher concentration of phytosterols dissolved therein. For example, the oil compositions used to prepare emulsions for spreads/margarines may have over 5% phytosterols. In this particular embodiment, it is preferred that one or more emulsifying agents be included in the composition as described below.

Emulisifying Agents

Optionally, one or more emulsifiers may be mixed with the phytosterols and/or phytostanols prior to the melting step. These emulsifiers include, but are not limited to (wherein bracketed numerals refer to the preferred HLB values): anionic surfactants such as alcohol ether sulfates, alkyl sulfates (30-40), soaps (12-20) and sulfosuccinates; cationic surfactants such as quaternary ammonium compounds; zwitterionic surfactants such as alkyl betaine derivatives; amphoteric surfactants such as fatty amine sulfates, difatty alkyl triethanolamine derivatives (16-17); and nonionic surfactants such as the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids and alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols, POE fatty

amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates and sorbitan esters. More specifically, the emulsifiers include: glycerin fatty acid esters, diglycerin fatty acid esters, polyglycerin fatty acid esters, organic acid glycerin fatty acid esters, propylene glycol fatty acid esters, sorbitan fatty acid esters and sucrose fatty acid esters. This list is not intended to be exhaustive as other emulsifiers are equally suitable. Most preferred as emulsifiers are lecithin and phospholipids. The addition of an emulsifier is most recommended wherein the concentration of phytosterols in the oil or fat composition is to exceed 5%, otherwise the addition should generally be unnecessary. It is preferred that the amount of emulsifier included be in the range of 0.01 to 10% w/w, more preferably in the range of 0.2 to 5% w/w.

Methods of Use

The oil and fat compositions of the present invention, comprising substantially completely dissolved phytosterols, may be used directly and without further modification in cooking, baking and the like as an agent to lower serum cholesterol in animals, particularly humans. Alternatively, the composition may be treated to enhance delivery into various other delivery media. For example, the present invention fully contemplates the formation of oleaginous gel foodstuffs such as peanut butter, mayonnaise, ice cream and margarine spreads incorporating such compositions. There are numerous modes or "vehicles" of delivery of this composition, accordingly, this invention is not intended to be limited to the following delivery examples.

1) Pharmaceutical Dosage Forms:

It is contemplated within the scope of the present invention that the composition of the present invention may be incorporated into various conventional pharmaceutical preparations and dosage forms such as tablets (plain and coated) for use orally, bucally or lingually, capsules (hard and soft, gelatin, with or without additional coatings) powders, granules (including effervescent granules), pellets, microparticulates, solutions (such as micellar, syrups, elixirs and drops), lozenges, pastilles, ampuls, emulsions, microemulsions, ointments, creams, suppositories, gels, and transdermal patches, modified release dosage forms together with customary excipients and/or diluents and stabilizers.

The composition of the present invention, adapted into the appropriate dosage form as described above may be administered to animals, including humans, orally, by injection (intra-venously, subcutaneously, intra-peritoneally, intra-dermally or intra-muscularly), topically or in other ways. Although the precise mechanism of action is unclear, the composition of the present invention, administered intra-venously, lowers serum cholesterol. It is believed that some blends of

phytosterols, in concert, may have, in addition to the role as an inhibitor of cholesterol absorption in the intestine, a systemic effect on cholesterol homeostasis through bile acid synthesis, enterocycte and biliary cholesterol excretion, bile acid excretion and changes in enzyme kinetics and cholesterol transport between various compartments within the body (PCT/CA97/00474 which was published on January 15, 1998).

The oil and fat compositions as described herein may be used in both dietary and therapeutic capacities in order to treat and/or prevent CVD, its underlying conditions such as hypercholesterolemia, hyperlipidemia, arteriosclerosis, hypertension, thrombosis, related diseases such as Type II diabetes, as well as other diseases that include oxidative damage as part of the underlying disease process such as dementia, aging, and cancer. In populations, which are considered "high-risk" for CVD or any of the oxidation related disorders, it is contemplated that the compositions and foodstuffs in which they are contained be used in primary, secondary and tertiary treatment programs.

In order to appreciate the various possible vehicles of the delivery of the compositions, the list below is provided. The doses of the compositions will vary depending upon, among other factors, the mode of delivery (i.e. how and into which food or beverage or pharmaceutical the composition is ultimately incorporated) , the patient size and condition, the result to be achieved, as well as other factors known to those skilled in the art of food additives and medicinal agents. Generally, however, it is preferred that the compositions of the present invention be administered to humans in a form comprising up to 6 grams (based on a 70kg person) of phytosterols and/or phytostanols per day, more preferably from 1-5 grams per day and most preferably 1.5 grams per day. It will also be recognized that the provision of much larger daily doses of the derivatives are not harmful to the animal host, as excess will simply pass through normal excretory channels.

Foods/Beverages/Nutraceuticals :

One primary purpose of the present invention is to create modified edible oil and fat compositions which can be used *per se* in cooking, frying and the like without further modification. Alternatively, the compositions of the present invention may be incorporated into or otherwise used in the preparation of foods, beverages and nutraceuticals, including, without limitation, the following:

1) Fat-Based Products--such as margarines, spreads, peanut butter, peanut spreads, mayonnaise (many of which are formed using emulsions), shortenings, cooking and frying oils and dressings;

2) Grain-based Goods--for example, bread and pastas, cookies, pastries, whether these goods are cooked, baked or otherwise processed;

3) Confectioneries--such as chocolate, candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, dairy and non-dairy shakes, icings and other fillings;

4) Beverages-- dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and any drinkable emulsions which contain added fat or oils;

5) Miscellaneous Products--including processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like; and

6) Dairy Products--butter, dairy spreads, and beverages such as shakes and any emlusions containing added fat or oils.

EXAMPLES

The present invention is described by the following non-limiting examples:

Example 1 Dissolution in Oils--Phytosterols in Soybean Oil

A number of compositions were prepared comprising phytosterols dissolved in oil, without the necessity of emulsifiers.

A) A mixture of phytosterols (hereinafter called "3P6") which comprises beta-sitosterol, campesterol, campestanol and sitostanol (the latter at about 33-40% w/w) was selected for dissolution in soybean oil. 0.5 grams of 3P6 (5% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.5 grams soybean oil (95%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

B) 0.6 grams of 3P6 (6% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.4 grams soybean oil

(94%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

C) 0.7 grams of 3P6 (7% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.3 grams soybean oil (93%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

D) 0.8 grams of 3P6 (8% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.2 grams soybean oil (92%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

E) 0.9 grams of 3P6 (9% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.1 grams soybean oil (91%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C

F) 1.0 grams of 3P6 (10% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.0 grams soybean oil (90%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

The 5% w/w phytosterol composition so formed yielded a clear solution with no discernible precipitation or crystals and was the most preferred composition without emulsifiers. Control sample compositions were prepared having comparable % ratios of the components, but made simply by adding the oil and phytosterols together with heat applied for approximately 3 minutes. As compared to the control samples, each of the compositions prepared by the method of the present invention was more clear indicating greater dissolution of the phytosterols..

Example 2: Dissolution in Oils --Phytosterols and Emulsifiers in Soybean Oil

Three types of emulsifiers were tested: EMULTOP ™(a lyso-PC enriched lecithin); EPIKURON 200™ (containing over 98% phospholipids) and Phosphoderm™ (approximately 80% phospholipids in alcohol). Each of these three were tested at amounts ranging from 0.01% w/w to 1% w/w. Phytosterol amounts ranged from 5-10% w/w. The protocol described below for

Epikuron at each set amount and phytosterols at 5% w/w is applicable to all compositons, substituting, of course, the different percentages of the three components

A) A mixture of phytosterols ("3P6" as described in Example 1) was selected for dissolution in soybean oil. 0.5 grams of 3P6 (5% w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. 0.1 grams of Epikurion 200 (1%w/w), an emulsifier with over 98% phospholipids was added and stirred into the melted phytosterols. After being well mixed, the phytosterol/phospholipids were added to 9.4 grams soybean oil (94%w/w) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

B) 0.5 grams of 3P6 (5%w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. 0.05 grams of Epikurion 200 (0.5%) was added and stirred into the melted phytosterols. After being well mixed, the phytosterol/phospholipids were added to 9.45 grams soybean oil (94.5%) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

C) 0.5 grams of 3P6 (5%w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. 0.03 grams of Epikurion 200 (0.3%) was added and stirred into the melted phytosterols. After being well mixed, the phytosterol/phospholipids were added to 9.47 grams soybean oil (94.7%) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

D) 0.5 grams of 3P6 (5%w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. 0.01 grams of Epikurion 200 (0.1%) was added and stirred into the melted phytosterols. After being well mixed, the phytosterol/phospholipids were added to 9.49 grams soybean oil (94.9%) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

E) 0.5 grams of 3P6 (5%w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. 0.001 grams of Epikurion 200 (0.01%) was added and stirred into the melted phytosterols. After being well mixed, the phytosterol/phospholipids were added to 9.499 grams soybean oil (94.99%) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

F) 0.5 grams of 3P6 (5%w/w) was heated to a molten condition under the heat of an oil bath at approximately 140° C. The molten phytosterol blend was added to 9.5 grams soybean oil (95%) at approximately 140° C. The final composition was mixed for about 2 minutes while the temperature was reduced to 100-110° C.

The best results i.e. clearest compositions with no discernible precipitation or crystals were obtained with 7% w/w phytosterol or less and 0.30% w/w lecithin (EMULTOP) as emulisifer and with 8% w/w or less phytosterol and 0.30% w/w phospholipids (PHOSPHODERM) in alcohol as emulsifier. As compared to control sample compositions (having comparable % ratios of phytosterols and oils without the emulsifiers); however, each of the compositions prepared by the method of the present invention and with emulsifier addition was more clear indicating greater dissolution of the phytosterols.

Example : 3 Dairy beverage

A phytosterol blend which consists of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in oil as per Example 1. Xantham gum (0.1%), skim powder milk (8-12%) were combined with skim milk and permitted to remain at room temperature for 30 minutes to re-hydrate powder milk. Next, a blend slowly mixed using an overhead stirrer such as Caframo equipped with a pitched blade impeller until uniform dispersion was obtained. Phytosterols containing oil was heated to 80 °C and added to the mixture while steering. Resulted mixture was than homogenized using a high sheer batch mixer (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). .). Other devices such as a single-stage homogeniser, a two-stage homogeniser or a high-pressure microfluidizer may alternatively be used for homogenization of the milk mix. Next, milk mix was submitted to UHT treatment (141 °C 4 sec) and packed in aseptic containers for use as a beverage or pasteurized (69oC, 30 min) for further processing.

This dairy beverage may be used as a "base" to prepare any number of food and beverage products. Although the preparation of yogurt is shown by way of example below, other products such as cheese may equally be prepared.

Example: 4 Yogurt

Pasteurized Phytrol containing dairy beverage (Example 3) was used to produced yogurt. Milk was standardized to 0.75 – 1% fat, 12 – 13% solids and 0.5-1% of the phytosterol blend using the Pearsons Square method (Hyde, K.A. and Rothwell, J., 1973, In Ice Cream, Churchill Livingstone

Ltd., London, U.K). About 3% by weight of active yogurt culture containing Lactobacillus bulgaricus and *Streptococcus thermophilus* in the ratio 1:1 were carefully introduced into warm milk mix. After gentle mixing, the inoculated milk was distributed into 125 g-containers filling to near top. The containers were thermally sealed with aluminum leads and placed in incubator (44°C) equipped with good uniform air circulator and temperature controller. Filled containers were permitted to remain at 44°C for 3-5 hours, until a firm, smooth gel was formed. During incubation, pH was monitored periodically. When pH reached about 4.5, yogurt was withdrawn from the incubator, chilled quickly and stored at 4°C.

Example: 5 Non-dairy beverage

A phytosterol blend which consists of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in oil as per Example 1. Xanthan gum (0.1 – 0.2%), Tween 65 (0.5-0.7%) and flavours were combine with water and slowly mixed using an overhead stirrer such as Caframo equipped with a pitched blade impeller at room temperature until uniform dispersion was obtained. Phytosterols containing oil was heated to 80 °C and added to aqueous mixture while steering. The mixture was homogenised and heat treated as described in Example 3.

Example : 6 Bread

Breads containing 0.6% and 1.2% of the phytosterol blend comprising campesterol, campestanol, β-sitosterol and sitostanol (hereinafter referred to as "Phytrol") dissolved in oil (Crisco ™) as per Example 1 were prepared using bread maker (Black & Decker, Model # B2005). The phytosterol composition (Crisco plus phytosterols) was mixed with the other ingredients in proportions indicated below.

Ingredients	0.6% Phytrol (g)	1.2% Phytrol (g)
Milk	334.00	334.00
Salt	7.50	7.50
Sugar	7.10	7.10
Crisco	12.00	12.00
Flour	535.00	535.00
Phytrol	5.42	10.84
Yeast	2.80	2.80

Ingredients were combined in the baking pan of bread maker. Preparation of dough and baking was conducting according the manufacturing instructions.

Example : 7 Cereal Bar

Cereal bars of total weight 20g, and 40g that contained 3%, and 1.5% of Phytrol, respectively, were prepared. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in partially hydrogenated vegetable oil using the protocol of Example 1. The oil/Phytrol blend was cooled to 30oC and emulsified using a high sheer batch mixer (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). Subsequently, two oil blends (9.4% and 18.8% of Phytrol) were further emulsified using a high-pressure microfluidizer at 20,000 PSI.

Cereal bars were produced by combining binder (40%), water (5%) and edible particles (55%). Below two typical examples of binder used for making a cereal bar.

Sucrose containing binder

Phytrol (9.4% or 18.8%) containing oil	40%
Sucrose	22%
Water	28%
Sodium Caseinate	5%
Lecithin	2%
Glycerin	3%

Glucose containing binder

Phytrol (9.4% or 18.8%) containing oil	40%
Glucose syrup	50%
Sodium Caseinate	5%
Lecithin	2%
Glycerin	3%

Sucrose in water /glucose syrup was heated to 100oC while Phytrol containing fat was liquefied at 40-80oC. Hot sugar solution was placed in the bowl (Hobart mixer, Model N50) and fat was added followed by adding all remaining binder ingredients. All ingredients were thoroughly and vigorously mixed. After cooling down to 40oC, edible particles are added while thorough, non-vigorous mixing was carried out. Following edible particles were typically incorporated into the cereal bars.

Edible particles

Rolled oats	20-40%
Crisped rice	10-20%
Puffed barley	10-20%
Dried apple dices	10-20%
Shredded coconut	5-10%
Raisins	5-10%
Various nuts	5-10%

After mixing was completed, mixed material was placed in the forming mold and pressed with a roller. After removal from the mold, it was cut into ready to eat various sizes cereal bars.

Example : 8 Spread

Light margarine (60% fat) containing 6% of Phytrol was produced in batches of 5-10kg. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in the oils using the protocol outlined in Example 1. Clear fat solution was placed in the feeding tank (20L), cooled to 40-45 oC and stirred using (Ultra-Turrax T50 equipped with the dispersing element S50N, IKA Works Inc., Wilmington, NC, USA). Next, the water fraction (40%) was added and temperature was adjusted to 60 oC. The blend was submitted into a votator and processed at 8-10oC. The composition of margarine is described below.

Ingredient	Wt%
Water Phase	
Water	39.0
Salt	1.0
Potassium sorbate	0.001
Oil Phase	
Soybean oil	38.025
Palm kernel oil	15.0
Phytrol	6.0
Mono/diglycerides	0.6
Lecithin	0.15
Flavor	0.075
Beta-carotene	0.15

Example : 9 Chocolate

Milk chocolate containing 6% of Phytrol was produced in batches of 20-50kg. Phytrol® consisted of campesterol, campestanol, β-sitosterol and sitostanol was dissolved in soybean oil using the protocol outlined in Example 1. The blend (20% Phytrol) was subsequently emulsified using a high-pressure microfluidizer at 20,000 PSI. Chocolate was composed of an outer shell (42 wt%, no Phytrol) and a center (69%, Phytrol). Chocolate outer shell was made by mixing sugar (45%), whole milk powder (20%), cocoa butter (23%), cocoa mass (12%), soy lecithin (0.3%) and pure vanilla (0.1%) in a heating tank. All ingredients were melted, tempered and deposited into molds. Center was prepare my mixing sugar, cocoa butter, whole milk powder, cocoa mass, soy lecithin and pure vanilla in the proportions as for outer shell. The mix was melted and tempered. Consequently, Phytrol/soybean oil blend was mixed with chocolate in the 1:1 ratio and deposited into molds previously filled with chocolate without Phytrol. Chocolate pieces were than cooled, wrapped and packed into the boxes. Using the molding system, 10-12 g chocolate pieces were produced.

Example: 10 Softgel Capsule Dosage Form.

Phytosterols were dissloved in an edible oil carrier using the protocol outlined in Example 1 and subsequently mixed with a dispersing/emulsifying agent and lecithin, in combination with a medium chain monoglyceride (di or triglycerides, or combinations thereof, may also be used). Depending on the purpose for which the combination product is used, the necessary dosage was supplied in one or two capsules taken with each meal.

Example: 11 Oral Microemulsion.

Phytosterols were dissolved in an edible oil using the protcol of Example 1 and then the composition was mixed with appropriate excipients to form a self-emulsifying drug delivery system which presented itself as a microemulsion in the gastrointestinal fluids. Suitable excipients comprised a blend of medium chain mono- and diglycerides having HLB values within the range 2-7, e.g. the CAPMUL (trademark) series; a medium chain triglyceride, e.g. a member of the CAPTEX (trademark) series; a high HLB emulsifier (HLB value 10-16), e.g. polysorbate 20 ; and water. Appropriate flavouring agents, preservatives and anti-oxidants were also incorporated. Depending on the purpose for which the combination product is used, the necessary dosage would be supplied in 5-10 mL of preparation taken with each meal.

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WE CLAIM:

1. A method of preparing a composition comprising an edible oil or fat and one of phytosterols and/or phytostanols and in which the phytosterols and/or phytostanols are substantially completely dissolved therein comprises the steps of:

a)heating the phytosterols and/or phytostanols to form a molten material;
b) heating the edible oil or fat;
c) mixing the molten material with the heated edible oil or fat; and
d) cooling the composition so formed.

2. The method of claim 1 wherein the oil is any edible oil of plant or animal origin and the fat is any animal fat.

3. The method of claim 1 wherein the oil is selected form the group consisting of sunflower oil, rapeseed oil, soybean oil, olive oil, corn oil, safflower oil, sesame seed oil.

4.The method of claim 1 wherein the phytosterols are selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

5. The method of claim 1 wherein the phytostanols are selected from the group consisting of sitostanol, campestanol, brassicastanol, desmostanol, chalinostanol, poriferastanol, clionastanol and all natural or synthesized forms and derivatives thereof, including isomers.

6. The method of claim 1 wherein the phytosterols and/or phytostanols are heated at step a) in the presence of an emulsifying agent.

7.The method of claim 7 wherein the emulsifier is selected from the group consisting of (wherein bracketed numerals refer to the HLB values): anionic surfactants such as alcohol ether sulfates, alkyl sulfates (30-40), soaps (12-20), sulfosuccinates; cationic surfactants such as quaternary ammonium compounds; zwitterionic surfactants such as alkyl betaine derivatives; amphoteric surfactants such as fatty amine sulfates, difatty alkyl triethanolamine derivatives (16-17); nonionic surfactants such as the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids, alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol,

octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols, POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates and sorbitan esters, lecithin, phospholipids, glycerin fatty acid esters, diglycerin fatty acid esters, polyglycerin fatty acid esters, organic acid glycerin fatty acid esters, propylene glycol fatty acid esters, sorbitan fatty acid esters and sucrose fatty acid esters.

8.The method of claim 1 wherein the phytosterols and/or phytostanols are heated at step a) to a temperature of between 120-145° C.

9. The method of claim 1 wherein the edible oil or fat is heated at step b) to a temperature of between 80-150° C.

10. A composition comprising an edible oil or fat and one or more phytosterols and/or phytostanols, wherein the phytosterols and/or phytostanols are substantially completely dissolved therein by a method in which the phytosterols and/or phytostanols are heated to form a molten material which is then added to a heated oil or fat and the composition so formed is cooled.

11. The composition of claim 10 wherein the oil is any edible oil of plant or animal origin and the fat is any animal fat.

12. The composition of claim 10 wherein the oil is selected form the group consisting of sunflower oil, rapeseed oil, soybean oil, olive oil, corn oil, safflower oil, sesame seed oil.

13. The composition of claim 10 wherein the phytosterols are selected from the group /consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

14. The composition of claim 10 wherein the phytostanols are selected from the group consisting of sitostanol, campestanol, brassicastanol, desmostanol, chalinostanol, poriferastanol, clionastanol and all natural or synthesized forms and derivatives thereof, including isomers.

15. The composition of claim 10 wherein the phytosterols and/or phytostanols are heated in the presence of an emulsifying agent.

16. The composition of claim 15 wherein the emulsifier is selected form the group consisting of (wherein bracketed numerals refer to the HLB values): anionic surfactants such as alcohol ether

sulfates, alkyl sulfates (30-40), soaps (12-20), sulfosuccinates; cationic surfactants such as quaternary ammonium compounds; zwitterionic surfactants such as alkyl betaine derivatives; amphoteric surfactants such as fatty amine sulfates, difatty alkyl triethanolamine derivatives (16-17); nonionic surfactants such as the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated fatty acids, alkyphenols, water-soluble polyethyleneoxy adducts onto polypropylene glycol and alkyl polypropylene glycol, nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxyethanol, polyethylene glycol, octylphenoxy-polyethoxyethanol, lanolin alcohols, polyoxyethylated (POE) alkyl phenols, POE fatty amides, POE fatty alcohol ethers, POE fatty amines, POE fatty esters, poloxamers (7-19), POE glycol monoethers (13-16), polysorbates and sorbitan esters, lecithin, phospholipids, glycerin fatty acid esters, diglycerin fatty acid esters, polyglycerin fatty acid esters, organic acid glycerin fatty acid esters, propylene glycol fatty acid esters, sorbitan fatty acid esters and sucrose fatty acid esters.

17. The composition of claim 10 wherein the phytosterols and/or phytostanols are heated to a temperature of between 120-145° C to produce the molten material.

18. The composition of claim 10 wherein the edible oil or fat is heated to a temperature of between 80-150° C prior to addition thereto of the molten material.

19. A food product comprising the composition of claim 10.

20. A beverage comprising the composition of claim 10.

21. A pharmaceutical product comprising the composition of claim 10.

22. A method of treating or preventing cardiovascular disease and its underlying conditions including hypercholesterolemia in an animal comprises administering to the animal the composition of claim 10.